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14. ABSTRACT

This project aims to develop a novel magnetic resonance spectroscopy (MRS) based approach to assess the aggressiveness of breast tumors, by identifying characteristic nuclear magnetic resonance signal for breast cancer stem cells (CSCs) and using it to quantify the number of CSCs in breast tumor. Stem like cancer cells were isolated from MCF-7 breast tumor by side population (SP) sorting. 1H-NMR spectrum analysis identified two characteristic peaks of 1.28 ppm and 1.96 ppm, for the SP cells but not the unsorted cells in vitro. Application of the spectral biomarkers in vivo is still unsuccessful.

15. SUBJECT TERMS

Cancer stem cells: Rare cancer cells that are responsible for the tumor initiation and propagation
Spectroscopic biomarker: Spectra peaks that are characteristic for the cells of interest

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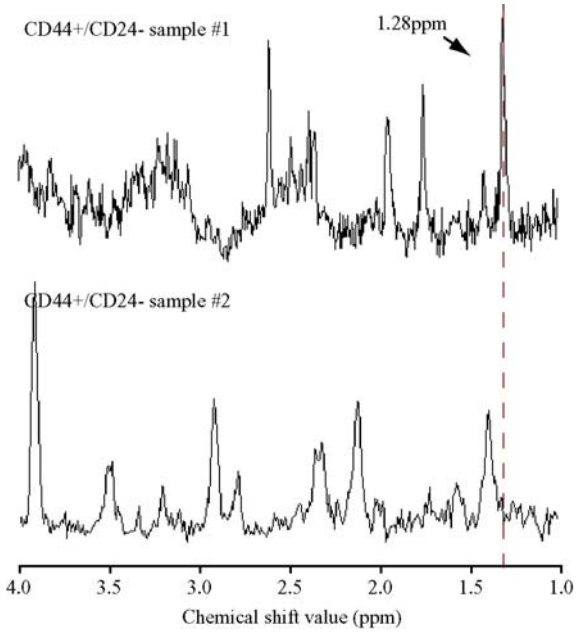
Introduction

Breast tumors are routinely graded according to differentiation levels in clinics. However, its application is limited because the method is invasive and not quantitative. Clinically 30%-60% of breast tumors are graded as moderately differentiated (grade 2) which is not informative for decision making [1]. Our hypothesis is that the number of cancer stem cells (CSCs) in a breast tumor can be used as an indicator for its aggressiveness. Our hypothesis is supported by increasing evidence reported in literature. Firstly, a recent study showed that poorly differentiated tumors are enriched with cells sharing characteristic with embryonic stem cells [2]. Independent studies also indicated that tumors characterized by aggressiveness feature may encompass a higher percentage of stem-like cancer cells than the less aggressive counterparts [3, 4]. Moreover, expression levels of biomarker genes for stem-like cancer cells have been observed to be reverse correlated with prognosis clinically [5, 6]. Based on the hypothesis, we aim to develop a novel magnetic resonance spectroscopy (MRS) based approach to assess the aggressiveness of breast tumors, by identifying characteristic nuclear magnetic resonance signal for breast CSCs and using it to quantify the number of CSCs in breast tumor.

Body

Task 1. Identify the ^{1}H-NMR spectroscopic biomarker for stem-like cancer cells

Tumor model was created by inoculating immunodeficient SCID mice with MCF-7 human breast cancer cells. Tumors were harvested and dissociated when they grew to about 1.5 cm in diameter. In order to identify ^{1}H NMR spectral peaks that are characteristic for breast CSCs, stem-like cancer cells were first isolated by flow cytometry as $CD44^{+}CD24^{-/low}Lineage^{-}$ cells from tumor specimen, the rest of the cells were designated as regular cancer cells and used as the control. Aqueous suspensions of cells were subjected to one-dimensional ^{1}H-NMR spectra analysis. The result spectra were compared to identify potential characteristic peaks for stem-like cancer cells. However, diverse spectra were got for different batches of cells isolated following the same cell sorting condition. In some batches we were able to identify the characteristic 1.28ppm signal for various progenitor cells [7], while in others we failed the detect the peak (**Fig. 1**).



The above result suggest that the sorted $CD44^{+}CD24^{-/low}Lineage^{-}$ cells are heterogenous, and may represent different types of cells for different tumors. We next sought to use different methods to isolate stem like cancer cells, aiming to purify a more homogeneous cell population. We adopted the widely used side population (SP) technique [8], in which stem like cancer cells are isolated as high dye-efflux cells. Our results (**Fig. 2**) showed that indeed a relatively constant percentage (~1.5%) can be isolated following Hoechst staining.

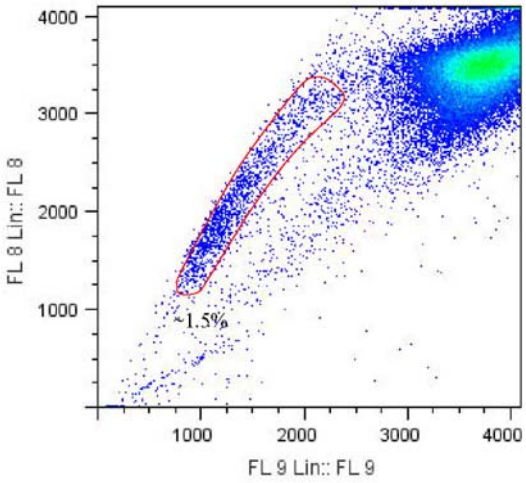
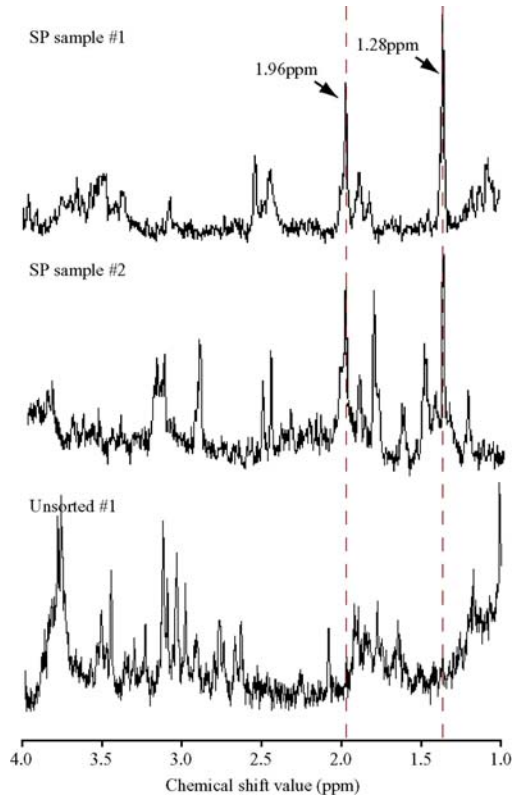


Fig. 2 SP cells (red boxed) isolated from the tumor. Cells were dissociated and stained with 5 μg/ml Hoechst 33342 for 90 minutes at 37°C. The Hoechst 33342 dye was excited at 357 nm and its fluorescence was analyzed under dual-wavelength of 402-446 nm (blue) and 650-670 nm (red).

We next carried out one-dimensional ^{1}H-NMR spectra analysis again on the aqueous suspensions of cells, either on SP sorted cells or unsorted cells. Our results showed that the 1.28 ppm stem cell characteristic peak can be repeatedly identified for the SP cells. A second peak of 1.96 ppm was also repeatedly dandified. Both peaks were undetectable in unsorted cells (**Fig. 3**).



Task 2. Test the potential of the biomarker for the quantification of stem-like cancer cells in vivo

Experiment was carried out to test the possibility of detecting the characteristic signal in vivo. Breast cancer tumor models were generated using three different methods, with the aim to create tumors with various contents of cancer stem cells. In model 1, tumors were generated by injecting regular MCF-7 breast cancer cells. In model 2, SP cells were isolated from MCF-7 cells and used for injection. In model 3, stem like cancer cells were selectively enriched by propagating MCF-7 cells in suspension as mammospheres, and the mammospheres were used for mouse injection. Animals were imaged with a 3T human MRI machine with a customized coil for small animals. However we were not able to detect either the 1.28 ppm or 1.96 ppm signal. Possible reasons were analyzed in the conclusion part.

Key Research Accomplishments

We were able to isolate SP cells from MCF-7 breast tumor, which are enriched with stem like cancer cells. ^{1}H-NMR spectrum analysis identified two characteristic peaks of 1.28 ppm and 1.96 ppm, which were repeatedly detected in SP cells but not in unsorted cells in vitro.

Conclusion

To identify characteristic NMR signals for stem like cancer cells, a relatively homogenous population of cells needs to be purified. We use two widely used FACS sorting methods to isolate stem like cancer cells from human tumors generated in mouse. Surprisingly, the most widely used $CD44^{+}CD24^{-/low}Lineage^{-}$ sorting generated a population that is very likely to be highly heterogeneous. And cells prepared from different tumors appeared to have different cell contents, as indicated by the highly diverse NMR spectra. Based on cellular dye-efflux function instead of a combination of cell surface markers, the SP sorting generated a more homogeneous and repeatable population. Two NMR peaks, 1.28 ppm and 1.96 ppm, were repeatedly identified for SP cells but not unsorted cells. These signals have the potential to be used as spectroscopic biomarkers for cancer stem cells.

The effort to apply the in vitro identified spectral biomarkers in vivo, however, was unsuccessful. Till now we were unable to detect either of the characteristic signals in live animals bearing xenografted tumor. The possible reasons are (1) in vivo cells have different cellular content from in vitro cultured cells, the molecules generating the characteristic signals are not indispensible for stem cells and are not expressed in vivo; (2) the ratio of cancer stem cells in a tumor is extremely low, the characteristic signal is undistinguishable from the background under current instrument sensitivity; (3) the signals detected from SP cells in vitro are not real biomarkers for CSCs, as there is no conclusive evidence that the SP population contains real CSCs, although it is suggested to be highly enriched with stem like cancer cells; (4) although our tumor models are generated from cells with different percentages of stem like cancer cells in vitro, the tumors generated do not necessarily have different stem cell contents, as it is well known that stem cells are under constant differentiation during tumor progression.

Recent report suggests that there are naturally occurring breast cancer subset highly enriched with cancer stem cells [9]. In the future it would be a better approach to compare the tumor samples naturally containing low cancer stem cell content and tumor samples naturally containing high cancer stem cell content (known as “claudin low”). Moreover, tumors generated by xenografting the clauding low tumor specimen can serve as a better model for high CSC containing tumor, therefore will greatly facilitate the in vivo validation of the biomarkers. It is also worth noting that even the signals characteristic for the claudin low sample are not generated from real CSCs, it does not affect its clinical application, as they still have the potential to be used as an indicator for the tumor aggressiveness, which is the purpose of this project.

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